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207 FLAVESCENCE DORÉE PHYTOPLASMA HAS MULTIPLE FTSH GENES DIFFERENTIALLY EXPRESSED IN PLANTS AND INSECTS

C. Jollard, X. Foissac, D. Desqué, F. Razan, L. Béven, S. Eveillard
UMR 1332 Biologie du Fruit et Pathologie, University of Bordeaux, INRA, Villenave d'Ornon, FRANCE

Background.Flavescence Dorée (FD) is a severe epidemic disease of grapevine caused by the FD phytoplasma (FDp) and is transmitted by the leafhopper vector *Scaphoideus titanus*. The recent deciphering of the 647 kbp-long FDp genome highlighted the unusual high number of genes encoding ATP dependent zinc proteases FtsH . Differences in virulence among isolates of 'C*andidatus* Phytoplasma mali' have recently been associated to variations in FtsH sequences. The aims of the present study were to predict the FtsH repertoire of FDp and the orientation of the various FtsH in the phytoplasma membrane, to measure the expression profile in different plant hosts and vectoring insects and to develop a heterologous expression system to sustain future studies of their associated ATPase and proteolytic activities. **Methods.** *Bacillus subtilis* and *Escherichia coli* FtsH sequences were blasted against the FDp predicted proteome. Sequences containing all FtsH functional motifs and representative bacterial and phytoplasmal FtsH were submitted to a phylogenetic analysis by maximum likelihood in MEGA6. FtsH topology was predicted using the Polyphobius software. Expression level of all *ftsH* genes was determined by SYBR Green quantitative RT-PCR in the natural grapevine/S. titanus and in the experimental broadbean/*Euscelidius variegatus* pathosystems. Expression profiles were normalized using the gyrA expression level. Finally, the soluble part of three FtsH were expressed in *E. coli* and purified and their ATPase and protease activities were measured using ATP and azocaseine as substrates. **Results.** Eight *ftsH* genes, namely *ftsh*1 to *ftsh*8, were identified along the FDp chromosomal sequence. All FtsH contained two *N*-terminal-transmembrane domains, the ATP-binding sites walkerA and walkerB, the SRH domain for the ATP hydrolysis, a zinc-binding site and an aspartate catalytic residue for proteolysis as well as the pore signal and the leucine-zipper involved in substrate binding. Beside FtsH6 that appeared to be the original ortholog of the conserved cellular FtsH, the other seven gene copies clustered on a common distinct phylogenetic branch, in agreement with an intra genome duplications of *fts*H. Interestingly, six ftsH genes were preceded by genes encoding secreted proteins homologous to solute-binding component of oligopeptide ABC transporter. All ftsH genes but one were expressed in the four different hosts tested. Relative Gene Expression (RGE) calculations showed that *fts*H3, 4 and 8 were overexpressed by FDp infecting grapevine as compared to their expression in the leafhopper *S. titanus*. In contrast, *fts*H1, 2, 6 were overexpressed by FDp infecting *S. titanus*. Gene *fts*H5 was highly and equally expressed in plants and insects.The FtsH *C*-tail was predicted by Polyphobius to be intracellular, except for FtsH6 and FtsH7 where it was predicted to be extracellular, therefore in direct contact with the host cellular content. The soluble part of three FtsH were expressed in *E. coli* and purified FtsH exhibited an ATPAse activity but failed up to now to exhibit a proteolytic activity. **Conclusion.** Eight *fts*H genes have been identified in the FDp genome that show host-dependent differential expression. Their involvement in the degradation of host proteins is still hypothetic.

Keywords: phytoplasmas, membrane proteases, protein metabolism

208 FROM COCONUT TO CASSAVA: THE COCONUT LETHAL YELLOWING PHYTOPLASMA IS WORSENING THE THREAT TO FOOD SECURITY IN CÔTE D'IVOIRE

Y. Arocha Rosete[1], K.D. Kra[2], M.N. Toualy[2], H.A. Diallo[2], J.L. Konan Konan[3], H. Montano[4], A. Bertaccini[5]
[1]Plant Pathology, Sporometrics Inc., Toronto, CANADA, ON, [2]Plant Protection, Université Nangui Abrogoua, Abidjan, CôTE D'IVOIRE, [3]Marc Delorme Station, Centre National de Recherche Agronomique, Abidjan, CôTE D'IVOIRE, [4]Plant Pathology, Federal Rural University, Rio de Janeiro, BRAZIL, [5]Dipsa, University of Bologna, Bologna, ITALY

Background. Cassava (*Manihot esculenta* Crantz) is a major staple food in the developing countries whose total world production reached approximately 278 million metric tonnes in 2017. Côte d'Ivoire produces around three million tonnes of cassava every year and reached 4.54 metric tonnes in 2017. It is typically consumed as 'attieké', which is currently exported on regional and international markets. Cassava crop is now threaten by the Côte d'Ivoire lethal yellowing disease (CILY) first reported and associated with a phytoplasma in Grand-Lahou in 2013. CILY destroyed over 400 ha of coconut groves in smallholder coconut farms where women farmers started planting cassava as an alternative food and cash crop in coconut lands devastated by the disease. **Methods.** Symptoms of leaf mosaic, curling and yellowing were observed in cassava orchards in

two coconut-growing villages located in the south coastal littoral of Grand-Lahou. Leaf samples were collected from symptom-bearing and symptomless cassava plants and subjected to total DNA extraction. PCR with phytoplasma universal 16S rRNA primers, and group-specific primers for subgroup 16SrXXII-B, '*Candidatus* Phytoplasma palmicola'-related strains; and with specific primers for African/Eastern cassava mosaic viruses (ACMV, EACMV). Amplicons were purified, cloned and sequenced. Sequences were compared to those of reference in NCBI (https://www.ncbi.nlm.nih.gov) and used for phylogeny analysis of phytoplasma and virus strains, respectively. **Results.** Phytoplasma DNA was amplified from six out of 12 symptom-bearing samples, five of which co-amplified virus DNA. Phytoplasma sequences showed 99% identity to those of 16SrXXII-B phytoplasmas as confirmed through phylogeny analysis. One cassava plant was co-infected with ACMV, closely related to the Angola strain, while the other four showed co-infection with both the ACMV (Angola) and an EACMV strain from Madagascar. All cassava varieties were phytoplasma-begomovirus co-infected, except the Yacé variety. **Conclusions.** Cassava plants in Grand-Lahou orchards were found infected by CILY phytoplasma (group 16SrXXII-B) and ACMV/EACMV virus strains. Results indicate that cassava may be an alternative host for the CILY phytoplasma, which may play a role spreading and worsening CILY epidemic. Prompt actions are required while waiting for a suitable resistant coconut cultivar. Short-term solutions may include replanting cassava yards with newly developed cassava varieties that enhance plant resilience against the coconut phytoplasma and ACMV/EACMV viruses to help supporting food production and improve livelihoods of smallholder coconut farmers in Grand-Lahou.

209 PHYLLODY INDUCTION IN DIVERSE PLANT SPECIES BY THE PHYTOPLASMA EFFECTOR PHYLLOGEN THROUGH DEGRADATION OF HOST FLORAL MADS-DOMAIN TRANSCRIPTION FACTORS

N. Iwabuchi[1], Y. Kitazawa[1], Y. Fujimoto[1], H. Koinuma[1], T. Nijo[1], T. Yoshida[1], Y. Okano[1], K. Maejima[1], Y. Yamaji[1], K. Oshima[2], S. Namba[1]
[1]Graduate School Of Agricultural And Life Sciences, The University of Tokyo, Tokyo, JAPAN, [2]Faculty Of Bioscience And Applied Chemistry, Hosei University, Tokyo, JAPAN

Background Phytoplasmas are economically important obligate plant pathogens in the class Mollicutes. Phyllody, in which floral organs become leaf-like structures, is a common symptom in phytoplasma-infected plants. ABCE-class MADS-domain transcription factors (MTFs) are key regulators of floral organ development in angiosperms. Aberrant expression of these genes can result in abnormal floral traits such as phyllody. Phyllogen, a virulence factor conserved in phytoplasmas, triggers phyllody in *Arabidopsis thaliana* (Brassicaceae) by inducing the degradation of A- and E-class MTFs. However, it is unknown whether phyllogen can induce phyllody in other species, despite the observation of phytoplasma-associated phyllody symptoms in a broad range of angiosperms. In this study, we investigated whether phyllogen can induce phyllody phenotypes in various plants. **Methods** To express phyllogen proteins of the onion yellows strain *Candidatus* Phytoplasma asteris and peanut witches' broom strain *Ca*. P. aurantifolia in a wide range of plants, we cloned these genes into the *Apple latent spherical virus* (ALSV) vector system, which can express foreign proteins in many plant species. *Agrobacterium* cells with ALSV-RNA1 and ALSV-RNA2 containing phyllogen and viral silencing suppressor P19 were co-infiltrated into leaves of diverse plants. To investigate whether phyllogen interacts with MTFs of diverse plants including gymnosperms and a fern, we performed a yeast two-hybrid assay. To determine whether MTFs were degraded in the presence of phyllogen *in planta*, YFP-fused MTFs were transiently expressed in *N. benthamiana* leaves by agroinfiltration in combination with either control GUS protein or phyllogen; to monitor their accumulation, we observed YFP fluorescence signals in the cell nuclei. Because the subcellular localization of each YFP-fused MTF was not limited to nuclei in all species, protein accumulation was confirmed by immunoblotting. Finally, to investigate the indirect effects of phyllogen on MTFs, we examined the expression levels of ABCE-class genes and flowering time genes in the floral buds of petunia plants affected by phyllogen. **Results** Phyllogen successfully induced phyllody in plants of the three different families (Solanaceae, Asteraceae, and Pedaliaceae), when expressed using the ALSV vector. Furthermore, it recognized and induced degradation of A- and E-class MTFs in two eudicots and in two monocots, as had previously been observed only in *A. thaliana*. The phenotypes of phyllogen-affected petunia flowers were very similar to those of E-class knockdown or knockout petunia mutants, and B- and C-class genes were downregulated in petunia buds expressing phyllogen; this expression pattern was similar to that of the E-class knockdown petunia mutant. In addition to recognizing MTFs from angiosperms, phyllogen recognized and induced degradation of MTFs from gymnosperms and a fern that are phylogenetically related to A- and E-class MTFs. **Conclusion** Phyllogen induces phyllody in angiosperms and inhibits MTF function in diverse plant species, functioning as a broad-spectrum virulence factor manipulating floral morphology. Downregulation of E-class MTFs, rather than A-class MTFs, was considered the main contributor to phyllogen-induced phyllody symptoms.